CIG final report for 'AMEMM' (CIG 630988)

Summary

This document outlines progress made under my four-year FP7 CIG 'AMEMM' (Grant Agreement Number 630988). The proposal as originally submitted described aims to understand the assembly, mechanism, and evolution of molecular machines in life on earth. Specifically, I was interested in studying the evolution of the bacterial flagellar motor – a rotary molecular motor just tens of nanometres across – as a case-study in understanding fundamental principles of how molecular machines have evolved in life on earth.

To accomplish this I proposed an approach combining 3D electron microscopy imaging to visualize the structure of the machinery, physical measurements to assess the mechanical output of the machinery, and molecular ancestry studies to contextualize these observations against a robust 'family tree' of molecular machines. Three broad aims were proposed: first, I described aims to develop methods to structural characterize molecular machinery, using the bacterial flagellar motor as the model system. Secondly, I described aims to relate the structures of these machines to their mechanical output. Thirdly, I outlined strategies to relate these results to phylogenetics of the component proteins towards understanding the mechanistic aspects of their evolution.

Scientific progress has been excellent, and I accomplished the majority of my aims for the project. I have published work on the structure of diverse flagellar motors and directly related these structures to their mechanical output, and published a follow-up study describing a possible evolutionary pathway to this diversity. I have recruited a postdoc to study the analogous archaellar motor with the intent of identifying evolutionary fundamentals to both archaellar and bacterial flagellar evolution, and have recruited postdocs to continue to study the diversity and evolution of flagellar motors.

As a result, my research career development has progressed extremely well. Since award of the CIG I have been awarded multiple additional pieces of grant funding in total providing over £1 500 000 of funds to my lab. I have published a number of papers which have received considerable press coverage from the mainstream media, and I have delivered, or am invited to deliver, over thirty talks on this work at major international conferences and institutions. I have recruited four postdocs and four PhD students. These successes have lead me to pass the probationary period at Imperial College and be awarded promotion to Senior Lecturer.

AMEMM Projects and accomplishments

Project 1: Method development in in situ structural dissection for mechanistic insights.

Building on previous successes, I proposed to develop methods to visalize molecular machines inside living ells, using the flagellar motor as a case study. Specifically, I sought to understand why, and how, bacterial flagellar motors have continued to evolutionarily diversify since emergence of the first flagellar motor.

Project 1 sought to develop methods for visualizing and interpreting 3D structures of bacterial flagellar motors. Toward this end I successfully determined the arrangements of the rotor and stators components of *Vibrio* and *C. jejuni*. Together this enabled us to construct a simple mathematical model that accounts for the torque produced by varied bacterial flagellar motors published in PNAS in 2016. Preliminary results from this contributed to successful award of a ~£500 000 BBSRC grant and recruitment of new postdoc to continue the project. I also continued collaboration with Dr David R. Hendrixson (UT Southwestern, Dallas, USA) to identify additional novel components of the *C. jejuni* flagellar motor that account for the higher torque generation. This resulted in Dr. Hendrixson being awarded an NIH R01 grant including approximately $100000 funds for my lab to cover data collection costs. We also made considerable progress on this project with PhD student Mr Teige Matthews-Palmer who, co-supervised with Prof. Willie Taylor (Crick Institute, London) published a manuscript in PLoS ONE describing predicted structures of all components of the type III secretion system. Mr Matthews-Palmer is now completing another manuscript describing the high-resolution structure of one of these components determined using electron microscopy.

Project 2: Relating structure of macromolecular machinery to mechanical output.

The diversity I demonstrated between flagellar motors posed an important question: what advantage do these organisms gain by altering a machine that works perfectly well for *E. coli*? I planned to develop an optical trap system in which we could directly measure flagellar motor torques.

The first steps toward this project were published in my 2016 PNAS publication, as discussed for Project 1. Toward direct measurement of torque we have constructed an optical device to perform the planned biophysical measurements in collaboration established with Dr Teuta Pilizota (U. Edinburgh). While this system is not operational as-yet, all hardware is now in place and we are awaiting a visit from Dr Pilizota this summer.

Furthermore, I have been awarded a three-year MRC research grant to fund alternative approaches in collaboration with Taka Nishizaka (Gakshuin U., Tokyo) and Wilson Poon (U. Edinburgh). This approach involves fluorescently labeling bacterial flagella for direct observation of rotation. The postdoc employed on this grant, Dr Eli Cohen, has now scheduled a three-week visit to the Nishizaka lab in Tokyo to drive data collection.

Project 3: Evolutionary mechanisms behind emergence of molecular machinery.

What evolutionary forces lead to already-functional proteins accreting to form rotary motors? While these processes are lost to billenia of evolution, *C. jejuni* and *Vibrios* are modern-day traces of an evolutionary pathway to progressively more complex motors. To understand how additional proteins were recruited, I planned to infer the ancestral states of motors by building a catalogue of the contemporary diversity of bacterial flagellar motors. Project 3 represented the heart of my interests and the core of my research programme. The PNAS paper mentioned above forms the foundation to build an evolutionary understanding of mechanisms underlying development of complexity in biological systems. Preliminary results from this paper provided the basis for the 3-year BBSRC grant that enabled me to hire Dr Bonnie Chaban and 3-year MRC grant to hire Dr Eli Cohen.

We have now published a first paper describing a model for the evolution of high torque, published in *Scientific Reports* in January 2018. We are now working to survey more diversity, have initiated crystalization experiments to determine the molecular structure of some of the recently evolved parts of bacterial flagellar motors, and I have now recruited a new PhD student to continue this project starting in October 2018.

Other contributions

Scientific

In addition to our work on the evolution of the bacterial flagellar motor, the AMEMM CIG facilitated pioneering collaborations to investigate the architecture of the bacterial periplasm. This culminated in publications in *Science* and *PLoS Biology.*

3D electron microscopy is viewed as a challenging technique, even in expensive ~ €5 000 000 electron microscopes. While the Imperial College microscope is capable of collecting high quality data, it is not designed for overnight data collection. I developed a roboticized workflow to automate data-collection on this microscope, enabling automated collection of an order of magnitude more data. A number of other international labs are now building their own roboticized data collection systems based on my work.

I have also been awarded a 'satellite' lab in the Crick Institute, located in central London. This enables me to position lab members embedded within another lab (the Rosenthal Lab) and gain access to the state-of-the-art electron microscopy equipment currently being installed at “The Crick”.

Transfer of knowledge

I have been of considerable benefit to Imperial College and the European community.

Training and mentorship: I have, or am, training three postdocs, three PhD students, approximately 15 MRes students and approximately 10 undergraduate students. This training and mentorship has focused on theory and practice of electron cryo-tomography, and a number of my students have already gone on to study for PhDs in electron cryo-microscopy labs where I have no doubt their background with me assisted their placement.

Teaching: I teach six hours of lectures of electron cryo-microscopy to final-year undergraduates every year, for which I am invariably rated extremely highly. Often my undergraduate project students became interested in my work after attending these lectures.

Societal impact

I am a passionate believer in scientific outreach to communicate our research to a wider audience. For the past four years my lab has directly engaged with the public at the three-day “Imperial Festival”, a science outreach festival for the general public to attend. Furthermore I have actively encouraged media engagement, and have received news articles on my research in the past year from New Scientist, Popular Science, Gizmodo, Popular Mechanics, Mental Floss, The Mail Online, Physics World, and (French popular science magazine) Science et Vie. I also recently published a more popular science take on our work in *The Biochemist*.